REMARKS

A check for the fee for a one month extension of time accompanies this response. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-12, 14, 15, 18, 19, 21-23, 27-28, 49 and 50 are pending in the instant application. Claim 1 is amended herein to clarify the subject matter of the claim and its dependents. Claim 1 is amended to clarify that the marker protein is used to identify or detect a target protein in a sample by virtue of the surface protein with which the which the marker protein is uniquely associated. Claims 2 and 3 are amended to correct minor obvious inconsistencies. Claim 21 is amended to depend from pending claim 1, which incorporates limitations of claim 20, which is cancelled herein. Claims 49 and 50 is amended to clearly reference antecedent basis in claim 1.

Claims 17 and 51 are cancelled to advance prosecution, and claim 20 is cancelled as it is no longer properly depends from claim 1, which specifies that the detection method is mass spectrometry. Applicant expressly reserves the right to file continuation applications to the cancelled subject matter.

The Examiner is thanked for the courtesy extended in granting an interview to clarify and confirm that the instant Office Action is **non-final** and that the indication on the cover sheet that the Action is final is an inadvertent error.

REJECTION OF CLAIMS 17 and 51 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claim 17 and 51 are rejected under 35 U.S.C. §112, first paragraph, as lacking enablement for the full scope of the claimed subject matter. Applicant does not agree with the basis for this rejection, since the marker proteins are reagents and it is routine to prepare such reagents with minor sequence variations. In the interest of advancing this application to allowance claims 17 and 51 are cancelled herein, thereby rendering this ground of rejection moot. Such cancellation should not be interpreted to limit the scope or content of the claims upon which claims 17 and 51, respectively depend.

REJECTION OF CLAIMS 1-12, 14, 15, 17-23, 26-28 AND 49-51 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-12, 14, 15, 17-23, 26-28 and 49-51are rejected under 35 U.S.C. §112, second paragraph as being indefinite. Various reasons for this rejection are provided, each of

which is addressed in turn below. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

As discussed previously, the amount of detail required to be included in the claims is not to be viewed in the abstract, but in conjunction with whether the specification is in compliance with the first paragraph of 35 U.S.C. § 112. If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (*Bendix Corp. v United States*, 600 F.2d 1364, 1369, 220 Ct. Cl. 507, 514, 204 USPQ 617, 621 (1979); *See, also, Carl Zeiss Stiftung v. Renishaw plc*, 20 USPQ2d 1094, 1101).

35 U.S.C. § 112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. It is unnecessary and unduly limiting to recite steps routine to those of skill in the art at the time the application was filed, such as wash steps and release of a bound polypeptide prior to analysis. A claim is not required to be a recipe, but rather defines the metes and bounds of the claimed subject matter. There is no requirement to include routine steps, which may be optional or performed in a variety of different ways.

Analysis

1) Claim 1 is rejected as being indefinite because the claim allegedly omits essential steps, such as a step of attaching a polypeptide binding component to a solid substrate, a wash step to remove unbound material, and a release step for releasing the polypeptide before the amplifying step. This rejection is respectfully traversed.

Claim 1 specifies that complexes of genetic packages with the target polypeptides are identified and the marker component is detected in genetic packages that have formed complexes. As amended, the claim further recite that the complexes are separated. Such separation, however, is not necessary requirement for mass spectrometric analysis, since components of complex mixtures can be identified by a predetermined molecular weight. Nevertheless, there are a variety of ways to separate complexes from a sample; reliance on a solid support is not necessary.

It is clear from the language of the claim to one of skill in the art that the complexes must be identifiable and, if necessary, separable from the uncomplexed material before

detection of the marker component. As discussed in great detail in the previous response, methods for identifying protein-protein complexes in a mixture containing unbound proteins and other material were well-known to those of skill in the art at the time the instant application was filed. Heterogeneous and homogeneous methods were known. It would be unduly limiting to require applicant to limit the claims to only heterogeneous methods. "[I]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter" Bendix Corp. v United States, 600 F.2d 1364, 1369, 220 Ct. Cl. 507,514, 204 USPQ 617, 621 (1979).

Definiteness of claim language must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of prior art and (3) the interpretation claims would be given by one possessing the level of skill in the pertinent art at the time the invention was made. The instant claims are definite because the steps of (a) contacting the sample with genetic packages such that complexes are formed by specific binding of the plurality of target polypeptides to polypeptide-binding components that are displayed on the genetic packages; and the genetic packages each contain a predetermined marker component that is indicative of its displayed polypeptide-binding component; (b) identifying complexes of the plurality of target polypeptides with the displayed polypeptide-binding components on the genetic packages; (c) optionally amplifying either the genetic packages that have formed complexes or the marker components that are in the genetic packages that have formed complexes; and (d) identifying marker components in the genetic packages that have formed complexes, thereby identifying a plurality of target polypeptides, adequately define the metes and bounds of the claimed subject matter in light of the disclosure in the specification and what is known to those of skill in the art.

The specification teaches that at the time of filing, several technologies were available for protein expression analysis and for analysis of complex mixtures of proteins, including protein-protein interactions (*see, e.g.*, page 2, line 15 to page 3, line 15), such as various immunodetection methods, direct visualization, two-dimensional gel systems and the use of protein-reporter gene conjugates. As incorporated by reference in the specification and made of record in the instant application, there clearly existed heterogeneous (*e.g.*, using a solid support) and homogeneous (single, *e.g.*, solution phase) methods of studying protein-protein interactions at the time the instant application was filed. Solution phase (homogeneous) methods do not require binding to a solid support for detection.

Furthermore, numerous methods for identifying and/or purifying protein-protein complexes in solution phase, including methods that do not require a wash step, were known. For example, phage-antigen complexes had been precipitated from a solution of complex protein mixtures by polyethylene glycol (PEG) precipitation, without non-specific protein contamination and without requiring a wash step (Telleman *et al.*, Biotechniques, 29(6):1240-1248 (2000); attached hereto). Similarly, Demartis *et al.* (J. Mol. Biol., 286(2):617-633 (1999); attached hereto) describes the isolation of phage-peptide complexes from solution by capture with magnetic beads or by PEG precipitation. As discussed above, other techniques including PEG precipitation, Sephacryl S-200 chromatography, DEAE-cellulose chromatography, Sepharose CL-2B chromatography, Q-Sepahrose chromatography, Fast Protein Liquid Chromatography (FPLC), gel filtration, affinity chromatography, velocity gradient centrifugation and native gel electrophoresis have been used to isolate and analyze protein complexes that are formed in solution. Additionally, homogeneous enzyme-based immunoassays were available in the art (in addition to ELISA assays) at the time of filing (Morris *et al.*, Ther. Drug Monit., 14(3):226-233 (1992); attached hereto).

Furthermore, in the interest of advancing prosecution, the claims now recite that the complexes are separated from the sample. The particular method for separation and treatment of complexes thereafter is well within the level of skill in the art, and as discussed above, a variety of methods for doing so were known at the time of the effective filing date of the pending claims.

. Therefore, it is respectfully submitted that one of skill in the art, given the methods as instantly claimed, would recognize (i) the scope/boundaries of the claimed subject matter; and (ii) the various techniques (heterogeneous and homogeneous phase) by which protein-protein complexes can be separated from a sample and identified e. No further elaboration in the claims is necessary to render the claims definite. A claim is not a recipe. It is respectfully submitted that in this instance, the claims reasonably apprise the skilled artisan of the scope and utilization of the claimed methods. Therefore, it is respectfully submitted that Claim 1 and claims dependent thereon are not indefinite.

2) Claim 2 is rejected as being vague and indefinite in the recitation of "disease-related protein," which is alleged to be unclear. The specification at page 11, lines 8-11, defines "disease-related protein" as follows:

> As used herein, [A] disease-related proteins refer to any polypeptides that are involved in or believed to be involved in diagnosis of a disease, the

cause of a disease, disease therapy, cure of a disease or other disease aspect or etiology.

Therefore, the meaning of "disease-related protein" is clear.

3) The rejection of claim 17 is moot by virtue of cancellation of claim 17 herein.

.REJECTIONS UNDER 35 U.S.C. §102(b)

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

"Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter is identically disclosed or described in the "'prior art'" . . .the [r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a §103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the *similarity* of the subject matter which he claims to the prior art, but it has no place in the making of a §102, anticipation rejection." (Emphasis in original). In re Arkey, Eardly, and Long, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

REJECTION OF CLAIMS UNDER 35 U.S.C. §102(b)

Claims 1, 2, 10-12, 18-20, 26, 49 and 50 are rejected under 35 U.S.C. §102(b) as being anticipated Larocca *et al.* (U.S. Patent No. 6,472,146), which the Examiner states

discloses methods for determining protein-protein interactions. The methods include contacting a sample with genetic packages that display a protein or peptide and that also include a reporter. Larocca *et al.* also discloses detecting the reporter as an indicator of complex formation. This rejection is respectfully traversed.

The rejected claims

Claim 1 is directed to a multiplexed method of detecting a plurality of target polypeptides in a sample. In the method, the sample is contacted with genetic packages that each display a polypeptide-binding component to form complexes between targets in the sample and displayed polypeptide-binding components that are specific for the targets. In addition to displayed polypeptide binding components, each genetic package includes predetermined marker component that is indicative its displayed polypeptide-binding component. Hence there is a different marker component for each displayed polypeptide. The marker component is different for each polypeptide-binding component so that identifying the marker identifies the polypeptide-binding component. Since each polypeptide-binding component is specific for a particular target, identification of a marker component detects a target polypeptide in the sample. After contacting, the claim now recites (the non-essential step) of separating complexes of the plurality of target polypeptides with the displayed polypeptide-binding components on the genetic packages from the sample; optionally amplifying the genetic packages that have formed complexes , resulting in amplified genetic packages, or amplifying the marker components in the genetic packages that have formed complexes. The marker components in the genetic packages in the separated complexes are then *identified* by mass spectrometry. Each marker component is uniquely associated with a binding component on the surface of a genetic package and thereby identifying the surface components, which specifically bind to target proteins As a result, target polypeptides present in the sample can be detected. Since there are a plurality of targets and different displayed polypeptides with different markers, the method can simultaneously identify a plurality of targets in a sample . Mass spectrometry, which identifies a marker by virtue of its molecular weight, is suited for such determination, since it is amenable to multiplexing and because it can identify a marker, not just detect it. Identification of the marker identifies the phage surface protein thereby detecting complexed proteins from the sample. Dependent rejected claims specify particulars of the method.

Disclosure of Larocca *et al*,

Larocca *et al.* discloses a ligand-displaying genetic package that carries a reporter or selectable marker and presents a ligand on its surface; the reporter is expressed only when the genetic package is internalized into a cell. The reporter reports when the genetic package has been internalized; it does ***not*** identify the displayed ligand.

The ligand-displaying genetic package is used in methods for *screening cells and tissues* for the ability to be transduced by the ligand displaying genetic package and also in methods for identifying internalizing ligands. The disclosed method of screening cells and tissues is practiced by a) contacting a library of ligand displaying genetic packages with a cell(s) or tissue(s), where each package carries a gene encoding a detectable product which is expressed upon internalization of the package into a cell or tissue; and (b) detecting product expressed ***by the cell(s) or tissue(s)***, and thereby identifying a target cell or tissue for internalizing ligands. Alternatively, the cells or tissues that express the reporter can be screened to identify the ligand that is internalized. Cells or tissues are screened by contacting a genetic package that expresses a potential ligand. Each package carries a gene encoding a detectable product, such as a reporter, that is expressed by the cells upon internalization of the package by a cell or tissue. In the methods for identifying internalizing ligands, nucleic acid encoding the ligand is isolated from the cells that internalize the genetic package and thereby express the reporter. Larocca *et al.* does disclose collections of genetic packages in which each marker component is different for each polypeptide-binding component.

Differences between the disclosed method of Larocca et al. and the rejected claims

The instant claims are directed to a multiplexed method of detecting a plurality of target **polypeptides** in a sample by contacting the sample with genetic packages that each display a polypeptide-binding component and that contain a marker that is indicative of the polypeptide binding component. Contacting is effected under conditions whereby target polypeptides in the sample form complexes with displayed polypeptide-binding components specific therefor. Each genetic package contains a predetermined marker component that is indicative of its displayed polypeptide-binding component and, hence polypeptides in the sample. The complexes are separated from the sample and the marker components are identified by mass spectrometry. Since each marker is associated with a unique polypeptide

binding component, identification of the marker identifies the binding component and thereby indicates which target polypeptides are present in the sample.

Thus, the methods of Larocca *et al.*. differ from the instantly claimed methods in several respects. (1) Larocca *et al*, discloses a method for screening a sample of *cells and target tissues* to identify which among them internalize a particular ligand. The instantly claimed methods are for identifying target polypeptides present in a sample, not screening cells and tissues to identify which among them internalize a ligand present on the surface of genetic package. In the instantly claimed methods, polypeptides are contacted with genetic packages; whereas, in the method of Larocca *et al.*, cells and tissues are contacted with genetic packages. (2) The genetic packages of Larocca *et al.* contain a reporter that is expressed upon internalization of the package into a cell. The genetic packages of the instantly claimed methods include a marker that is indicative of displayed binding polypeptide; the marker is not indicative of internalization. (3) In the methods of Larocca *et al.*, after contacting the genetic packages with the cells and tissues, cells and tissues that internalize a genetic package express the reporter. The cells and tissues are screened to identify which among them internalizes a genetic package. The cells and tissues, identified because they express the reporter In the instantly claimed methods, complexes of the polypeptide and genetic package are separated from the sample, and the marker components in tehin the genetic package are identified by mass spectrometry. Each marker is different for each genetic package that expresses a different polypeptide binding component. Mass spectrometry permits identification of a marker.

Larocca *et al.* does not disclose a method in which complexes of a genetic package and a polypeptide are separated from a sample and a marker protein is identified by mass spectrometry. Larocca *et al.* identifies cells and tissues that express a reporter. Larocca *et al.*, does not disclose use of mass spectrometry to identify a marker. In the methods of Larocca *et al.*, the reporter does not differ for each displayed binding polypeptide. (4) The method of Larocca *et al.* is a method for identifying cells and tissues that internalize a ligand and/or for identifying a ligand that internalizes into cell; the instantly claimed methods are for identifying polypeptides in a sample. (5) Larocca *et al.* does not disclose collection of packages in which the reporter component in the genetic package is uniquely associated with the polypeptide-binding component on the surface of the genetic package.

Larocca *et al.*, does not disclose a method for identifying polypeptides in a sample; does not disclose genetic packages that contain a binding protein indicative of the polypeptide binding component; does not disclose formation of and separation of complexes of any sort, including complexes of the binding component on the surface of the genetic package and a polypeptide (the genetic packages are contacted with cells and are internalized; Larocca *et al.* does not identify a marker component by mass spectrometry. Thus, Larocca *et al.* fails to disclose a method for identifying polypeptides in a sample that includes the steps of contacting the sample with genetic packages that contain a marker indicative of the polypeptide binding component on their surfaces, separating complexes of the genetic complexes and polypeptides in the sample, and identifying a marker in the genetic package by mass spectrometry, it does not anticipate any pending claims. Therefore Larocca *et al.* does not anticipate any of the rejected or pending claims.

Rebuttal to the Examiners allegations

1) The Examiner urges that Larocca *et al.* discloses a method for determining protein-protein interactions and that it can be multiplexed.

Larocca *et al.* discloses a method for identifying ligands that internalize and/or cells or tissues that internalize ligands. Larocca *et al.* does not disclose a method for identifying proteins in a sample. The instant claims are not directed to methods for determining protein-protein interactions per se, but to methods for detecting the presence of proteins in a sample. This is very different from a method for screening for cells or tissues that internalize a ligand or for identifying internalizing ligands.

In the method of Larocca *et al.* the phage are screened by a pool and then split method to identify which one expresses an internalizing ligand on its surface. Cells are contacted with pools of phage; and if the pool contains a phage that expresses an internalizing ligand, the internalized reporter gene is express **by the** cells. The pool is then split and each new pool tested until the phage that expresses the internalizing ligand is identified., In contrast, in the instant methods, proteins not a phage, in a sample, that form complexes with binding proteins on the surface of the genetic package are identified. The marker is not expressed in a cell, but is identified by mass spectrometry.

With respect to multiplexing, Larocca *et al.* at col. 11 states that a variety of fluorescent proteins are known so that a plurality of different reporters can be used simultaneously. Larocca *et al.*, however, does not disclose that each binding protein is

associated with a different reporter. In the multiplexing method, a plurality of different phage that express internalizing ligands can be identified simultaneously; this is not the same as identification of a plurality of proteins in a sample. This is different from a method as claimed in the instant application, in which each different binding protein on the surface of a phage is associated with a unique marker so that a plurality of different proteins in a sample can be identified..

2) The Examiner urges that Larocca *et al.* discloses a detecting a predetermined marker component to detect complexes between genetic packages and proteins or peptides in a sample. It is respectfully submitted that this is not correct. As noted, in the method of Larocca *et al.* the cells that internalize ligands are detected by looking for expression of a reporter gene that is expressed upon internalization of a ligand into the cell. Protein-genetic package complexes are not detected; internalized genetic packages are detected. The method does not detect proteins in a sample.

Furthermore, the instant claims require that each genetic package comprises a predetermined marker component that is indicative of its the displayed polypeptide-binding component; and the polypeptide-binding component specifically binds to at least one of the target polypeptides, whereby target polypeptides that bind thereto can be detectged by virtue of identification of the marker protein. Larocca *et al.* does **not** disclose a method in which each genetic package comprises a predetermined marker component indicative of the displayed polypeptide-binding component. The genetic packages contain nucleic acid encoding a reporter that is expressed by a cell when the genetic package is internalized; expression of the reporter is indicative of internalization, not of the particular polypeptide binding component.. Each genetic package does not contain a reporter that is indicative of the displayed polypeptide binding component. Larocca *et al.* does not disclose such relationship. Larocca *et al.* does disclose that cells that express the reporter are screened to identify the ligand component , which would not be necessary if each ligand binding component was associated with a different marker. Furthermore, in the method of Larocca *et al.* the identity of the ligands expressed on the surface of the cell is not known; and methods are provided to identify the ligand component. In the instantly claimed methods, each polypeptide binding component is associated with a predetermined marker, and the methods identify proteins in a sample, not the polypeptide binding components.

3) As discussed above, Larocca *et al.* discloses a method for screening for cells and tissues in a sample that internalize ligands and/or for identifying internalizing ligands expressed on the surface of the genetic packages. The instantly claimed methods identify proteins in a sample; the method of Larocca *et al.* does not; it screens for cells or tissues in the sample, or identifies the ligands expressed on the surface of the polypeptide binding component.

Therefore, Larocca *et al.* does not disclose the instantly claimed methods and does not anticipate any pending claims.

REJECTION OF CLAIMS UNDER 35 U.S.C. §103(a)

Relevant Law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritsch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesch, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified,

then the teachings of the references are not sufficient to render the claims prima facie obvious. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

1) Claims 1-11, 14, 15, 18-20, 22, 23, 27 and 28 are rejected as being obvious over the combination of teachings of Georgiou (U.S. Patent No. 5,866,344) in view of Larocca *et al.* because Georgiou teaches competitive immunoassay methods in which an antibody is displayed in the surface of a host cell, such as a bacterium, which is then contacted with a sample containing an analyte under conditions that favor complex formation. The immunoassay involves contacting the antibody display bacteria with labeled analyte, followed by competitive displacement of the bound labeled analyte with a known amount of unlabeled analyte and measuring the unbound label, thereby allowing quantitation of binding affinity. The Examiner urges that the only difference between the teachings of Georgiou and the instantly claimed methods is that Georgiou fails to teach multiplexing. The Examiner cites Larocca *et al.* provides this teaching. This rejection is respectfully traversed.

The claims

As noted the instantly claimed methods are multiplex methods for identifying proteins in sample in which a phage library, where members express particular polypeptide-binding component, is contacted with the sample to form complexes between the proteins and phage. Each phage contains a marker that is indicative of the polypeptide-binding component. Upon formation of complexes, the particular polypeptide components, and hence polypeptides in the sample, are detected by identifying the associated markers by mass spectrometry. The instant methods can simultaneously detect a plurality of target polypeptides by complexation with polypeptide-binding components on genetic packages, where each polypeptide-binding component is associated with a unique predetermined marker component and the marker components are detected simultaneously by mass spectrometry.

Teachings of the cited references and differences from the instant claims

Georgiou

Georgiou teaches an immunoassay method for quantitating a particular analyte in a sample. Georgiou teaches a method in which expression libraries are prepared such that the each protein is displayed on the surface of a cell, such as a bacterial cells. In these methods, the proteins expressed on the cells surface *i.e.*, the displayed polypeptide, are the proteins that are being screened, not proteins in a sample. They are screened by contacting them with a labeled target antigen , and cells that express a protein that specifically binds to the antigens

are identified. This method is very different from the instantly claimed methods in that the genetic packages (the cells) do not include a marker that is unique to the displayed polypeptides; the identities of the displayed polypeptides is unknown. Furthermore, the method is not for identifying proteins in a sample, but rather for screening a library by expressing proteins encoded by the library on the surfaces of cells.

Georgiou also teaches whole cell immunoassays in which cells that express a particular antibody are used in competitive immunoassays to **quantitate the amount of an** analyte in a sample. In practicing the assays Georgiou states (col. 7, lines 25-50):

> . . . a known amount of the antibody-covered cells are placed in a solution of a known concentration of the analyte-conjugate along with an unknown concentration of the analyte (the test solution). The analyte conjugate competes with free analyte in solution for binding to the antibody molecules on the cell surface. The higher the concentration of analyte conjugate in the solution, the fewer molecules of fluorescein analyte conjugate bind on the surface of the cells, and vice versa.
>
> The mixture is centrifuged to pellet the cells, and the fluorescence of the supernatant is measured. The assay is quantitative because the amount of observed fluorescence is proportional to the concentration of analyte in the test sample, *i.e.*, if there is a very low concentration of analyte to compete with the fluorescein conjugate, then most of the conjugate will bind to the cells and will be removed from solution. The more molecules of analyte in solution, the more molecules of analyte bind to the antibodies thereby preventing the conjugate from binding. In this case, more fluorescein conjugate remains in the supernatant to give a stronger fluorescence signal. The assay can be calibrated to generate a quantitative measurement of the unknown concentration of analyte. The entire assay requires less than one hour. Fluorescence determinations may be made with a basic fluorimeter.

The Examiner urges that the "surface-expressed polypeptides may also be antibody conjugates that include detection proteins." This is not correct. In the methods of Georgiou, a known quantity of analyte is labeled. The analyte conjugates are conjugate of analyte and a label, not a surface protein. Since these are competitive assays, free label in solution after binding analyte to the antibody-expressing bacterial cells is proportional to the amount of analyte. This is very different from a method for detecting polypetides in a sample as in the instant claims.

Further, even if the method of Georgiou included surface-express polypeptides that include detection proteins, which is not correct, such method would be different from the instantly claimed methods, in which each genetic package includes a marker that is indicative

of the polypeptide binding protein displayed on the surface of the package. There is no suggestion in Georgiou for use of a label indicative of the displayed polypeptide.

Hence this method of Georgiou differs from the instantly claimed method in a variety of aspects, including: 1) The method is a quantitative competitive binding method in which the *amount* of an analyte is determined. This is very different from the instantly claimed methods in which the presence of particular target is detected. 2) Georgiou does not teach or suggest the inclusion of a marker in its cells that express the surface protein, nor a marker that is indicative of the antibody on the surface. 3) The method of Georgiou does not detect a marker in the cell (the genetic package) to identify a protein in a sample, but detects the presence of fluorescence in the medium resulting from unbound labeled analyte that competes with the unknown amount of analyte in the sample. Unbound fluorescent label is detected. Analyte is not detected, but is quantitated. The identity of the analyte is known. The instantly claimed method is a method for detecting a plurality of proteins in a sample by contacting a genetic package that expresses a surface protein and includes a marker indicative of the surface protein with a sample. The marker is identified by mass spectrometry to thereby identify the protein. These steps are absent from the teachings of Georgiou.

Larocca *et al.*

The teachings of Larocca *et al.*, discussed above, fail to cure these deficiencies. The method of Larocca *et al.* is a method for identifying cells and tissues that internalize a ligand and/or for identifying a ligand that internalizes into cell; the instantly claimed methods are for identifying polypeptides in a sample. As discussed above, Larocca *et al.* does not teach or suggest a method in which complexes of a genetic package and a polypeptide are separated from a sample and a marker protein is identified by mass spectrometry. Larocca *et al.* teaches identification of cells and tissues that express a reporter when a genetic package bearing an internalizing ligand is internalized into the cells . Larocca *et al.*, does not teach detection of a marker component in the genetic package nor its identification by mass spectrometry. In the methods of Larocca et al., the reporter does not differ for each displayed binding polypeptide. Larocca *et al.* does not teach or suggest a collection of packages in which the reporter component in the genetic package is uniquely associated with the polypeptide-binding component on the surface of the genetic package; the reporter component is expressed upon internalization of a genetic package bearing an internalizing ligand on its surface.

Therefore the combination of teachings of Georgiou with those of Larocca *et al.* does not result in or suggest the instantly claimed methods

The combination of teachings of Georgiou with those of Larocca *et al.* does not teach or suggest a method nor result in a method for detecting or identifying a plurality of proteins in a sample. The method of Georgiou is a competitive immunoassay for quantitating an analyte or a method for screening antibodies expressed on the surface of a genetic package. The method of Larocca *et al.* is a method for screening cells and tissues to identify cells that internalize a ligand and/or to identify internalizing ligands on a genetic package. The instantly claimed method is for detecting a plurality of proteins present in a sample.

Further neither Greorgiou nor Larocca *et al*, singly nor in any combination thereof, teaches or suggests a method in which a genetic package includes a marker that is associated with the expressed surface protein, whereby detection of a marker in a complex between a protein in the sample and the genetic package identifies a surface protein and thereby identifies a polypeptide in the sample. In addition, neither reference, singly nor in any combination thereof, teaches mass spectrometric detection of marker proteins that are in genetic packages the complex with proteins in a sample to identify proteins in the sample. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

2) Claim 21 is rejected under 35 U.S.C. §103(a) as being unpatentable over Georgiou and Larocca *et al.* in view of Hutchens *et al.* because Georgiou and Larocca *et al.* allegedly differ from the method of claim 21 in failing to teach or suggest detection by matrix-assisted laser desorption/ionization (MALDI), which deficiency is allegedly taugh by Hutchens *et al.*, which teaches detecting genetic packages by MALDI. The Examiner concludes that:

> It would have been obvious to one of ordinary skill in the art to incorporate matrix-assisted laser desorption/ionization mass spectrometry for detection as taught by Hutchens *et al.* into the modified method of Georgiou because Hutchens *et al.* teaches that this provides for the simultaneous mass screening of very large numbers of genetic packages bearing different polypeptides and Georgiou specifically teaches that [its] method is readily adaptable to the determination of multiple analytes. Thus one of ordinary skill in the art would have had a reasonable expectation of success incorporating MALDI into the modified method of Georgiou.

This rejection is respectfully traversed.

The claims

Claim 1, which is not subject to this rejection is discussed above. It has been amended, in the interest of advancing prosecution to recite that the markers are identified by

mass spectrometry. Hence all claims include this requirement. Claim 21recites that the mass spectrometric format is MALDI.

Teachings of the cited references and differences from the claimed methods

The teachings of Georgiou and Larocca *et al.* are discussed above. It is presumed for this discussion that the "modified method of Georgiou" refers to the combination of teachings of Georgiou and Larocca *et al.* discussed by the Examiner in the rejection addressed above. As discussed above, the combination of their teachings is deficient in numerous respects. As stated above, the combination of teachings of Georgiou with those of Larocca *et al.* does not teach or suggest a method for detecting or identifying a plurality of proteins in a sample. The method of Georgiou is a competitive immunoassay for quantitating an analyte or a method for screening antibodies expressed on the surface of a cell. The method of Larocca *et al.* is a method for screening cells and tissues to identify cells that internalize a ligand and/or to identify internalizing ligands. The instantly claimed method is for detecting a plurality of proteins in a sample. Since neither reference teaches or suggests s method for detecting a plurality of proteins in a sample, the combination thereof cannot do so.

Further neither Greorgiou nor Larocca *et al*, singly nor in any combination thereof, teaches or suggests a method in which a genetic package includes a marker that is associated with the expressed surface protein, whereby detection of a marker in a complex between a protein in the sample and the genetic package identifies a surface protein and thereby identifies a polypeptide in the sample. In addition, neither reference, singly nor in any combination thereof, teaches mass spectrometric detection of marker proteins that are in genetic packages the complex with proteins in a sample to identify proteins in the sample.

Hutchens *et al.*

Hutchens *et al.* fails to cure any of these deficiencies. Hutchens *et al.* does not teach a method for detecting proteins in a sample; it provides a method for screening polypeptides displayed on the surface of a genetic package. Hutchens *et al.* does not teach or suggest a method in which genetic packages containing markers that are indicative of the polypeptide are employed nor a method in which the markers in complexes between genetic packages and proteins in a sample are identified in order to detect or identify proteins in sample. on the surface are detected in order to are identified

Hutchens *et al.* teaches (paragraph [0049]):

> . . . a method of detecting a genetic package containing a polynucleotide that *encodes a polypeptide agent that specifically binds to a target* adsorbent.

This is, in one aspect, a combinatorial method for selecting analyte-specific phage from a display library, including the use of target proteins isolated by retentate mapping or target proteins generated in situ by in vitro transcription and translation The method comprises the steps of: a) providing a substrate comprising a target adsorbent; b) providing a display library that comprises a plurality of different genetic packages, each different genetic package comprising a polynucleotide that comprises a nucleotide sequence that encodes a polypeptide agent, and *each different genetic package having a surface on which the encoded polypeptide agent* is displayed; c) exposing the substrate to the display library under elution conditions to allow specific binding between a polypeptide agent and the target adsorbent, whereby a genetic package comprising the polypeptide agent is retained on the substrate; and d) *detecting a genetic package* retained on the substrate by desorption spectrometry.

Hutchens *et al.* does teach that MALDI is a method for desorbing and/or ionizing analytes for direct analysis are well known in the art. Hutchens *et al.*, however, teaches a method for detecting *genetic packages* that form complexes with a target to which a displayed surface polypeptide binds. It is the surface proteins that are detected, not proteins in the sample. This is not a method for identifying a plurality of polypeptides in a sample, but is rather a method for screening displayed surface proteins, Furthermore, Hutchens *et al.* does not teach or suggest a method in which genetic packages include a marker that is *indicative* of the displayed polypeptide and then identifying the marker to detect polypeptides in a sample as is instantly claimed. Thus, Hutchens *et al.* does not teach identifying marker proteins by mass spectrometry; Hutchens *et al.* identifies displayed polypeptides.

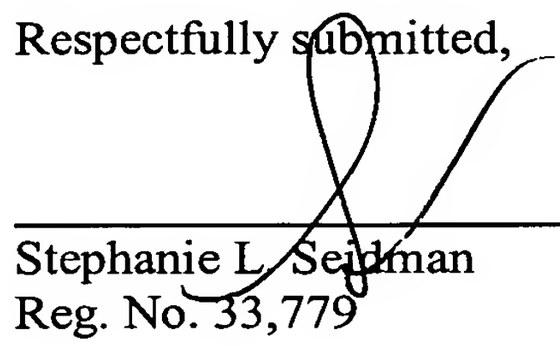
Therefore, Hutchens *et al.* does not cure the deficiencies in the methods of Georgiou and Larocca *et al.*, singly nor in any combination thereof. Neither Hutches *et al.*, nor any cited reference singly or any any combination thereof, teaches or suggests a method for detecting or identifying a plurality of proteins in a sample; nor does it, nor any cited reference singly or any any combination thereof, teach or suggest a method in which a genetic package includes a marker that is associated with the expressed surface protein, whereby detection of a marker in a complex between a protein in the sample and the genetic package identifies a surface protein and thereby identifies a polypeptide in the sample. Furthermore, neither Hutchens *et al. n*or any cited reference singly or any any combination thereof teaches or suggests mass spectrometric detection of marker proteins that are in genetic packages in a complex with proteins in a sample to identify proteins in the sample. Therefore, the combination of teachings of Georgiou and Larocca *et al.* and Hutchens *et al.* fails to result in

the instantly claimed methods. Hence the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the above, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,



Stephanie L. Seidman
Reg. No. 33,779

Attorney Docket No. 17111-008001/2308
Address all correspondence to:
Stephanie L. Seidman
Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130
Telephone: (858) 678-5070
Facsimile: (202) 626-7796
email: seidman@fr.com